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#### **Season and size effects: changes in the quality of gilthead sea bream (***Sparus aurata* **L.)**

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#### **Abstract**

The aim of this study was to investigate the potential effects of the harvesting season and fish size on the final quality of cage-farmed' gilthead sea bream (*Sparus aurata*). Morphometric analysis, fat store estimation, fillet composition analysis, fillet fatty acids profile determination and sensory analysis were carried out on a commercially-sized cage farmed gilthead sea bream (*Sparus aurata* L.) population in April, June, July and November.

Both season and size had a significant effect in morphometric characteristics. The most pronounced size effect was the change of fish shape, becoming rounder with fish growth, the increase of filleting yield and condition index. An increase of both peritoneal and perivisceral fat was observed in summer months. In general, size had little effect on muscle composition. A significant size effect was observed in the fillet lipid content, which increased with it. Fillet lipid content was unexpectedly lower in the warm period (June-July). This can be possibly attributed to feeding restrictions that may have occurred, due to anoxic incidents related to high temperatures.

Slight seasonal differentiations occurred in the muscle fatty acids. No significant differences were found in the organoleptic characteristics of the two size groups compared.

**Keywords:** Fat depots; Fillet composition; Sea bream; Season; Size; Quality.

#### **Introduction**

Fish consumption is strongly recommended by health authorities due to its high ˆ-3 polyunsaturated fatty acid (PUFA) content, playing an important role in the prevention of cardiovascular diseases (SCHMIDT *et al.,* 2005). An increasing percentage of fish consumption is of fish of aquaculture origin,aquaculture having experienced a boom in the last decade (FAO, 2006). The latest efforts in aquaculture focus on the farming of new species and on the improvement of product quality and safety (FAO, 2006).

Farmed fish quality in production terms is related not only to the achieved growth, but also to the final characteristics of the fish, including its flesh composition and nutritional value, its organoleptic characteristics and its general acceptance.

Most of the studies concerning the final quality of common Mediterranean species like the sea bream (*S. aurata*) are related to its freshness and shelf life (LOUGOVOIS *et al.,* 2003; CHOULIARA *et al.,* 2004), growth (COMPANY *et al.,* 1999), rigor mortis (BAGNI *et al.,* 2006) or nutritional characteristics (IZQUIERDO *et al.,* 2005; TESTI *et al.,* 2006; FERREIRA *et al.,* 2007) as affected by handling, feeding, killing and postmortem storage conditions. However, very little knowledge exists of the effects of the season on the muscle fatty acid profile (SENSO *et al.,* 2007) and on its general quality and somatometric characteristics (GRIGORAKIS *et al.,* 2002). Furthermore, these studies refer to fish of the same size produced at different seasons, i.e. they refer to different batches of fish with the same dietary history or origin. No data exists on how the muscle quality, the somatometric characteristics and fat depots of the same population change seasonally. Therefore, the aim of this study was to investigate the quality changes of a commercially-sized cagefarmed, gilthead sea bream population, in order to outline the potential effects of season and size on the final product quality.

# **Materials and Methods**

# *Rearing conditions and samplings*

Sea bream (*S. aurata*) of 65 g initial body weight were reared in  $12 \times 12 \times 10$  m<sup>3</sup> cages in a fish farm in the Saronic Gulf (Poros is-

land, Greece), with a growing density of 10-  $12 \text{ kg/m}^3$  for a period of 14 months. Fish were fed with a commercial feed at a concrete ratio based on feeding tables provided by the aquafeed company. The feed gross composition and fatty acid profile is presented in Table 1.

Fish were sampled from various cages of the same unit at various commercial sizes, and in four different sampling seasons (April, June, July and November). Water temperature in sampling periods was 15, 20, 25 and 18° C in April, June, July and November respectively (Table 2).

In total, 112 sea bream were killed by immersion in ice-water slurry, packed with flaked ice into polystyrene boxes and carried to the laboratory within 3-4 hours of harvesting. Samples of fish were kept at  $4^{\circ}$  C in a domestic refrigerator while sampling was performing.

Morphometric analysis, fillet composition analysis, fillet fatty acids profile determination and sensory analysis were conducted. The number of the individuals analyzed in each case is presented in Table 2.

# *Morphometric analysis, fat depots and fillet proximate composition*

Total body weight, standard length, measured from the mouth to the base of the caudal fin, height, taken at the pectoral fin vertical line and maximum width, measured at the height of the pectoral fin, were determined in every fish. After dissection, liver, gonads, perivisceral fat (the fat stored inside the peritoneal cavity) and peritoneal fat (the fat located around the peritoneal cavity) were weighed.

Condition index (CI) were determined according to the following formula  $CI = 100 \text{ x W (g)} / \text{Lst}^3 \text{ (mm)}^3$  where W is the fish total body weight and Lst its standard length.

Peritoneal fat index, perivisceral fat index, hepatosomatic index (HSI), gonadosomatic index (GSI) and fillet yield, were calculated as percentages of the total body weight.

Fillet proximate composition analyses took place according to the official methods of AOAC (1998).

#### *Fillet fatty acids profile determination*

Samples for muscle fatty acid analysis were extracted from the front dorsal part of the fillet in a chloroform methanol (2:1 v/v) solution, within 7-8 hours after slaughter. Pools of total 1g tissue each were made from 3-4 different fish.

The fatty acids were extracted according to FOLCH *et al.* (1957) and the methyl esters were obtained as described by CHRISTIE (1989). The methyl esters were separated and quantified by gas chromatography with flame detector, using helium as carrier gas as described by FOUNTOULAKI *et al.* (2003). A standard fatty acid mixture (Supelco 37 component FAME mix) was used as a pattern and the data were analyzed using the Millenium Software. The feed methyl esters were obtained directly from a 50-70 mg of ground sample as described by LEPAGE & ROY (1984).

Atherogenicity index (AI) and thrombogenicity index (TI) were determined by using the formulae:  $AI = (12:0 + 4 \times 14:0$  $+ 16:0$  / ( $\Sigma MUFA + \Sigma \omega 6 + \Sigma \omega 3$ ) and  $TI = (14:0 + 16:0 + 18:0) / [0.5)$  MUFA +  $0.5\Sigma\omega 6 + 3\Sigma\omega 3 + (\Sigma\omega 3/\Sigma\omega 6)$ , where  $\Sigma MUFA$  is the sum of mono-unsaturated fatty acids,  $\Sigma \omega$ 3 the total n-3 fatty acids and  $\Sigma\omega$ 6 the total n-6 fatty acids respectively.

#### *Sensory analysis*

In July, 10 sea bream were additionally sampled in order to compare the sensory quality of different sizes. Two different

# **Table 1 Feed composition and fatty acids profile as % of the total fatty acids.**



size groups were examined for their possible organoleptic differences (fish of <250 g and fish of 350 g). Within 8-10 hours of harvesting, fish were gutted, filleted and whole fillets were wrapped in aluminium

<b>Analysis</b>	April	June.	<b>July</b>	<b>November</b>
Size range $(g)$	126.0-323.9	207.8-374.1	172.2-376.3	369.2-500.3
Size average $(g)$	210.3	281.7	271.5	431.5
Morphometry	30 fish	30 fish	30 fish	10 fish
Composition	30 fish	30 fish	30 fish	10 fish
Organoleptic	٠	-	20 fillets	$\overline{\phantom{a}}$
Fatty acids*	5 pools		5 pools	3 pools

**Table 2 Size ranges, size averages, and total samples analysed in every sampling.** 

\*Pools for fatty acid analysis were made of 3-4 fishes.

foil and steam cooked for 20 min. Quantitative Descriptive Analysis (QDA) (MURRAY *et al.,* 2001) was used to evaluate flesh taste, flavour, colour, oiliness, elasticity, hardness, stickiness and general acceptance. Ten assessors previously having received the training required for QDA (MURRAY *et al.,* 2001) conducted the sensory test. A 0 to 5 scale was used for rating the intensity of the characteristics.

#### *Statistical analysis*

Regression analysis against body weight was performed for each of the studied parameters within each season, and slopes were compared. In cases of non-significant weight effect, one-way analysis of variance (ANOVA) was used to find the effects of season. In cases of significant weight effects and parallel regression lines (i.e. similar effect for all seasons), ANCOVA with weight as covariance was used for group comparisons. In cases of significant weight effect and non-parallel lines, ANCOVA comparisons were made only for the season groups that were parallel, since comparisons among non-parallel lines do not make sense (RAUBEHEIMER & SIMPSON 1992).

Regression analysis was done by Statgraphics and analysis of variance by SPSS

13.0 software. The Tukey test was used to determine the possible significant differences among values ( $p<0.05$ ). Data from sensory analysis were analyzed by a non parametric Kruskal-Wallis test.

#### **Results**

Both season and size had a significant effect on morphometric characteristics of the studied fish. Morphometric characteristics altered by fish size in the June and July samples, i.e. in the summer period (Table 3). Thus, length/height and length/width were found to decrease with fish size within summer samplings, while condition index increased.

Table 4 shows the morphometric characteristics of the studied population at the different sampling seasons. Regarding the season, there was no significant effect on condition index, while length/height and length/width were found significantly increased in the July sampling.

Filleting yield increased with fish size for July fish, but remained unaffected by size in the rest of the samplings. Significant differences were also found in HSI and GSI with respect to the fish size, increasing with it, in November and April, respectively.

# **Table 3**

**Characteristics of regression lines describing morphometric characteristics and quality indexes in relation to body weight.** 



(continued)

	Intercept	<b>Slope</b>	$*{\bf R}^2$	Sig <sup>1</sup>	% explain of the variability
<b>Perivisceral Fat Index</b>					
April	4.0946	$-0.082b$	$-0.311$	ns.	9.7
June	$-1.2654$	0.0014a	0.545	**	29.7
July	0.1832	0.0112a	0.545	**	29.7
N <sub>ov</sub>	$-2.1225$	0.0109a	0.561	ns	31.5
<b>Total Fat Index</b>					
April	4.6080	$-0.0083b$	$-0.2946$	ns	8.7
June	$-0.6803$	0.0162a	0.548	$***$	30.1
July	$-0.3403$	0.0167a	0.652	**	42.5
<b>Nov</b>	$-1.346$	0.0104ab	0.500	ns	25.

**Table 3 (continued)**

Different letters  $(a, b, c)$  stand for statistically significant differences ( $p < 0.05$ ) between sampling seasons 1Significance level of the regression (ns = no significant;  $\binom{*}{p}$  < 0.05;  $\binom{**}{p}$  < 0.01)

\*Correlation coefficient

# **Table 4 Morphometric characteristics, quality indexes of the studied population at the different sampling seasons.**



Different letters  $(a, b, c)$  stand for statistically significant differences  $(p \le 0.05)$ . 1Standard error of the means.  $N =$  Number of samples.

Significant size effect for perivisceral and total fat depots, increasing with it, was found only in summer samplings and for peritoneal fat in the July sampling (Table 3).

Seasonal significant differences were found in most of the quality indexes. Periv-

isceral and total fat depots were higher during the summer (June-July) and peritoneal fat was also significantly higher in June. Average peritoneal fat content in July seems also to be higher than in the spring and autumn, but this sampling could not be included, since a different size-effect was observed for this sampling (non-parallel regression line).

Size did not affect muscle protein (Table 5), but affected muscle fat, which increased with fish size in April and July. Muscle moisture and ash decreased with size in July but remained unaffected by size in the rest of the samplings ( $Table 5$ ).

With respect to fillet composition, protein was significantly higher during the summer (June and July) whereas lipid percentage was significantly lower in the same season. Moisture and ash content were found higher in June than in the rest of the samplings (Table 6).

The fatty acid profiles of the flesh are shown in Table 7. In all cases, the predominant fatty acids among the saturated ones (SFA) were myristic acid (14:0), palmitic acid (16:0) and stearic acid (18:0). Palmitoleic acid (16:1  $\omega$ 7) and oleic acid (18:1  $\omega$ 9)

	Intercept	<b>Slope</b>	$\mathbb{R}^2$	Sig <sup>1</sup>	% explain of the variability
<b>Muscle Fat</b>					
April	3.4069	0.0158	0.439	$**$	19.3
June	2.0674	0.0059	0.284	ns	8.1
July	1.3684	0.0130	0.411	$\ast$	16.9
<b>Nov</b>	8.4160	$-0.0016$	$-0.043$	ns	0.2
<b>Muscle Protein</b>					
April	19.7389	0.0013	0.089	ns	0.8
June	21.8147	$-0.0011$	$-0.107$	ns	1.1
July	20.9919	0.0014	0.1488	ns	2.2
<b>Nov</b>	19.5390	0.0016	0.1470	ns	2.2
<b>Muscle Moisture</b>					
April	67.2890	$-0.0040$	$-0.012$	ns	0.0
June	74.3742	$-0.0062$	$-0.313$	ns	9.8
July	76.0066	$-0.0168$	$-0.539$	**	29.1
<b>Nov</b>	70.1536	0.0004	0.011	ns	0.0
<b>Muscle Ash</b>					
April	1.4228	0.0000	0.054	ns	0.3
June	1.5336	$-0.0001$	$-0.125$	ns	1.5
July	1.5216	$-0.0004$	$-0.389$	$\ast$	15.2
<b>Nov</b>	1.3730	0.0000	0.063	ns	0.4
Energy (Kcal/g)					
April	1.2123	0.0011	0.335	ns	11.3
June	1.0841	0.0005	0.278	ns	7.7
July	0.9792	0.0013	0.459	$\ast$	21.1
<b>Nov</b>	1.7206	$-0.0005$	$-0.147$	ns	2.1

**Table 5 Characteristics of regression lines describing fillet composition in relation to body weight.**

<sup>1</sup>Significance level of the regression (ns = no significant;  ${}^*p$  < 0.05;  ${}^*p$  < 0.01)

# **Table 6**

# **Muscle composition of the studied population at the different sampling seasons ± standard error of the means.**



Different letters (a, b, c) stand for statistically significant differences (p <0.05)  $^*N$  = Number of samples.

### **Table 7**

# **Seasonal changes of fillet fatty acids (expressed as % of the total fatty acid content ± standard error of the means).**



were the predominant fatty acids among the mono-unsaturated ones (MUFA). Among poly-unsaturated fatty acids (PUFA), the most abundant were linoleic (18:2  $\omega$ 6), EPA  $(20:5 \omega 3)$  and DHA  $(22:6 \omega 3)$ . Concerning the global percentages, the most abundant were PUFAs (with  $\Sigma_{0}3$  predominating among them), followed by MUFAs (mostly  $\Sigma_{09}$ ). Table 7 shows the seasonal variations in the fillet fatty acid profile of the studied population. ∑SFA was significantly higher during the summer, due to the significantly higher content of 14:0.

∑MUFA were found higher in November. The  $18:1 \omega$ 9 content was lower in summer than in autumn and spring. With regard to PUFAs,  $\Sigma\omega$ 6 were found significantly lower in the November sampling, mostly due to the lower  $18:2 \omega 6$  content, and ratio  $\Sigma \omega 3/\Sigma \omega 6$  was found higher at this season. Among the  $\omega$ 3 fatty acids, the EPA percentage was higher in the summer than in the rest of the samplings.

With regard to the atherogenicity and thrombogenicity indexes (AI and TI respectively), only AI was found significantly higher in summer.

Sensory analysis did not show significant differences between the two studied size groups (Fig. 1).

### **Discussion**

For most of the studied parameters (length/height, length/width, condition index, fillet yield, peritoneal, perivisceral and total deposit fat, fillet fat, moisture, ash and energy) the size effects were most profound in summer, mostly in July and to a lesser degree in June, than in the other samplings (Tables 3 and 5). This could be possibly due



*Fig. 1:* Sensorial scores of the descriptors for the studied size groups.

to the higher size ranging within the summer samplings and especially in July (Table 2).

Morphometric characteristics in our experiment were within the normal values for that species, although rearing conditions, i.e. growing density and feeding, can affect the morphological characteristics (FLOS *et al.,* 2002).

The facts of significant length/height and length/width reduction, as well as the significant increase with size (in June and July) of the condition index (Table 3), constitute serious indications that in commercial sizes the main fish growth is due to a gain in weight, while fish become more round-shaped.

In the present study condition index did not show significant seasonality (Table 4). A seasonality in condition index has been mentioned for Atlantic Salmon Salmo salar (YOUNG *et al.,* 2006), with highest values observed at the end of the warm period (in September) and a reduction observed thereafter during winter. However, condition index seasonality has been found to be dietarily affected by the dietary energy content (SOLBERG, 2004; YOUNG *et al.,* 2006), while positive correlation with fish size (YOUNG *et al.,* 2006) and influence of other dietary treatments such as food deprivation (GRIGORAKIS & ALEXIS, 2005) can further complicate a possible seasonal effect in condition index.

The average filleting yield in the present study (33.2%) remained slightly lower than the range of  $34.3 - 48$  % mentioned in literature (GRIGORAKIS & ALEXIS, 2005; TESTI *et al.,* 2006). Filleting yield increased with size only in July samples. The higher size heterogeneity for July fish compared to the rest of the samplings (Table 2) gives a possible explanation for significant size effect only for this sampling. Size effect explains why the present average filleting yield was found slightly lower than that of

the literature, since the present study also included a large number of smaller fish (150 – 250 g). The significant increase of filleting yield with size, also poses the question of finding the most profitable sizes for gilthead sea bream filleting.

Gonadosomatic index was lower during the summer (June, July) and increased in November, but in all cases remained very low. A similar seasonality without reaching maturity has been previously described for gilthead sea bream by GRIGORAKIS *et al.* (2002).

Hepatosomatic index had its maximum value in April and decreased in summer. Liver lipid depot increase with water temperature drop has been previously mentioned by IBARZ *et al.* (2005) as a mechanism of adaptation to the cold. However, the reason for HSI summer reduction can also be due to dietary factors, and this will further be analysed.

Total depot of fat, considered as the sum of peritoneal and perivisceral fat indexes, is comparable to that previously found (GRIGORAKIS *et al.,* 2002; SANTINHA *et al.,* 1999). The seasonality observed for fat depot accumulation, being higher in the warm months (June and July) partially agrees with what was found by GRIGORAKIS *et al.* (2002) who also found increased fat depots at the end of the summer, but also for fish sampled later in the autumn.

The presently found average values for muscle protein  $(20.8\%)$ , fat  $(5.8\%)$ , moisture  $(71.3\%)$  and ash  $(1.4\%)$  are within the common limits reported for this species (FLOS *et al.,* 2002; GRIGORAKIS *et al.,* 2002; ÖZYURT *et al.,* 2005; SENSO *et al.,* 2007).

Fillet protein content and fillet lipid content showed an opposite trend. A significant increase in fillet protein content was found during the summer together with

a significant fillet lipid content decrease. Protein content stability at commercial sizes, with no seasonal or dietary variations has been mentioned by previous studies (GRIGORAKIS *et al.,* 2002; ÖZYURT *et al.,* 2005; SENSO *et al.,* 2007). This contradicts the present results where a seasonal effect was observed with protein found to be higher in summer fish.

Also a seasonal change was observed for muscle fat, but contrary to the literature which found either muscle fat increase during summer (GRIGORAKIS *et al.,* 2002) or no specific muscle fat trend throughout the year (SENSO *et al.,* 2007). The fact of such different seasonal trends indicates the sensitivity of gilthead sea bream muscle fat to dietary and environmental conditions. A possible explanation of low fat in the summer period could be due to muscle fat mobilization because of high metabolic needs in summer not met by the dietary intake. The low HSI values in the summer months (Table 4) could possibly indicate the same thing. Liver and muscle fat have been mentioned as the first fat depots mobilizing in the gilthead sea bream (GRIGORAKIS & ALEXIS, 2005). Insufficient dietary energy intake could be probably explained due to a frequent summer phenomenon in Greek aquaculture, where high water temperatures in combination with low levels of dissolved oxygen, often demand restriction of feeding.

Muscle lipid content and muscle moisture content showed an opposite trend, which is in accordance with the literature (WEATHERLY & GILL, 1983).

The fatty acid profile of the present study is in general typical for farmed fish species. However, saturated fatty acids were found slightly lower than the usual range of 26 – 31 % mentioned in the literature (GRIGORAKIS *et al.,* 2002; SAGLIK *et al.,* 2003; IBARZ *et al.,* 2005; IZQUIERDO

*et al.,* 2005; VASILIADOU *et al.,* 2005; TESTI *et al.,* 2006; MNARI *et al.,* 2007), but similar to the levels mentioned by SENSO *et al.* (2007). Mono-unsaturated fats are within the values of  $30 - 37$  % mentioned in the literature (GRIGORAKIS *et al.,* 2002; SAGLIK *et al.,* 2003; VASILIADOU *et al.,* 2005; TESTI *et al.,* 2006; MNARI *et al.,* 2007). As in our study, in all of the previously mentioned literature, palmitic acid (16:0) is the predominant saturated fatty acid, while oleic acid  $(18:1\omega9)$  predominated among the mono-unsaturated ones. Regarding  $\omega$ 3 poly-unsaturated fatty acids, their total levels were found similar to those of SENSO *et al.* (2007) and MNARI *et al.* (2007), but higher than those mentioned in previous studies (GRIGORAKIS *et al.,* 2002; SAGLIK *et al.,* 2003; VASILIADOU *et al.,* 2005; TESTI *et al.,* 2006). The literature showed a great variability in  $\omega$ 3/ $\omega$ 6 ratios, ranging from 1.47 (IBARZ *et al.,* 2005) to 4.09 (MNARI *et al.,* 2007), and present results are within this range. Also the total EPA and DHA levels are in accordance with GRIGORAKIS *et al.,* 2002; SAGLIK *et al.,* 2003; IBARZ *et al.,* 2005; IZQUIERDO *et al.,* 2005; VASILIADOU *et al.,* 2005; TESTI *et al.,* 2006; MNARI *et al.,* 2007; SENSO *et al.,* 2007.

Seasonal effects on the fillet fatty acid profile of cultured gilthead sea bream has rarely been studied (SENSO *et al.,* 2007). In general fish fatty acid changes due to the season, mainly include an increase in the unsaturation level (primarily in the polar lipid fraction) as a response to the cold, in order to maintain the appropriate fluidity of biological membranes (HAZEL & PROSSER, 1974; DELGADO *et al.,* 1994). In gilthead sea bream, an increase in unsaturation in white muscle, with artificially applied temperature drop, has been mentioned (IBARZ *et al.,* 2005). An increase of DHA at low temperatures has been mentioned in a seasonal study of wild gilthead sea bream (ÖZYURT *et al.,* 2005). Our study, however, failed to show such a seasonal impact similar to the other existing source in cultured gilthead sea bream (SENSO *et al.,* 2007). Higher SFA levels in summer were also observed in our study, but were not followed with a respective decrease in unsaturates (Table 7). The existing data for wild gilthead sea bream (ÖZYURT *et al.,* 2005) also failed to show an increase in the total unsaturated fatty acids in colder periods (winter). In any case, fatty acid mobilization in wild fish is expected to be different, since maturation and spawning also play an important part in this process (ÖZYURT *et al.,* 2005), unlike in their farmed counterparts.

The atherogenicity index is the ratio between the main saturate and the main unsaturated fatty acids, and the higher its value, the higher the tendency of lipids to adhere to cells of the immunological and circulatory systems. The average value (0.39) was within the range of 0.25 (SENSO *et al.,* 2007) and 0.49 (AMERIO *et al.,* 1996) found for the same species. The present study indicated that AI is seasonally variable. However, the only existing previous reference to sea bream did not find significant seasonal variations in AI (SENSO *et al.,* 2007).

The thrombogenicity index is an indicator of the clot forming tendency in the blood vessels. According to our results, TI does not appear to depend on the season. Similarly SENSO *et al.* (2007) did not show any seasonal effect on TI. Since limited respective data occur in literature regarding AI and TI season and size effect, further research would be useful.

One of the most important aspects for consumers is the sensory characteristics of the fish. Organoleptic characteristics mainly depend on the fillet composition and its

fatty acid profile and both of them are strongly affected by dietary factors (IZQUIERDO *et al.,* 2005). Results on sensory analysis, not exhibiting any organoleptic differences since no significant differences were found, correlated with size in the fillet composition and were minor in the fatty acid profile.

# **Conclusion**

Results in the present study showed that the quality of the cage-farmed sea bream fluctuates with the harvesting season. The most profound seasonal effect was the increase of deposit fat (both perivisceral and peritoneal) during the summer period. A minor seasonal effect was observed for muscle fatty acids. On the other hand, the size did not affect fillet protein content or organoleptic quality of the fish but had some significant positive effects on fillet fat. Size mainly affected the morphometric characteristics of the fish. In particular there is an indication the fish become more roundshaped with growth. Some quality indexes which are important for fish consumers, such as the condition index, fillet yield or peritoneal fat and total deposit fat, increased with size.

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